IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner: **Art Unit:** **Docket No.: 1896**

In RE: **Application of V. PATCHEV, et al**

Title: **In vitro screening for ligands of the estrogen receptor**

Ser. No.: **-**

Filed: **Simultaneously**

November 21, 2001

SIMULTANEOUS AMENDMENT

Hon. Commissioner of Patents

and Trademarks,

Washington, D.C. 20231

Sir:

Simultaneously with the filling of the above-identified U.S. Patent Application, please make the following changes and consider the following REMARKS:

In the Claims:

Please replace claims 1 to 3 with the following amended claims 1 to 3 and add the following claims 4 to 9:

1(amended). A method of *in vitro* screening for a ligand including selecting said ligand by means of at least two assay systems, said method comprising the steps of:

a) in a cellular or tissue assay system comprising an estrogen receptor and an estrogen receptor-driven reporter gene, selecting the ligand having a transcriptional activity mediated by activation of the estrogen receptor and measured by detecting a potency in the cellular or tissue assay system, whereby in the cellular or tissue assay system the ligand activates the potency with a half-maximally effective ligand concentration ($EC_{50(ER)}$) less than or equal to 10 nmol/l, and

detecting the activation of the transcription;

and

b) in a cell-free or enzymatic assay system, selecting a physical-chemical interaction of a co-present steroid receptor coactivator-1, and fragments thereof, and the estrogen receptor, which is measured by detecting a potency of said interaction in the cell-free or enzymatic system,

wherein the ligand activates the estrogen receptor and induces said interaction with said co-present steroid receptor coactivator-1, and

maximally effective ligand concentration ($EC_{50(ER+SRC)}$) greater than or equal to 100 nmol/l, and detecting a potency of the physical - chemical interaction of the co-present steroid receptor coactivator-1, and fragments thereof, and the estrogen receptor.

2(amended). A method of *in vitro* screening for a ligand, said ligand being an estrogen or having estrogenic activity, in a cell-free or enzymatic assay system by selecting a physical-chemical interaction of a co-present steroid receptor coactivator-1, and fragments thereof, and an estrogen receptor, said physical-chemical interaction being measured by detecting a potency of said interaction in the cell-free or enzymatic assay system,

wherein the ligand activates the estrogen receptor and induces said interaction with the co-present steroid receptor coactivator-1, and fragments thereof, in the cell-free or enzymatic assay system with a half-maximally effective ligand concentration ($EC_{50(ER+SRC)}$) greater than or equal to 100 nmol/l, and detecting the potency of the physical-chemical interaction of said co-present steroid receptor coactivator-1, and fragments thereof, and of said estrogen receptor.

3(amended). A method of *in vitro* screening according to claim 2, wherein said ligand is said estrogen and transcriptionally activates a cellular assay system comprising said estrogen receptor and an estrogen-receptor-driven reporter

gene, wherein the ligand activates a potency with a half-maximally effective ligand concentration ($EC_{50(ER)}$) less than or equal to 10 nmol/l.

4. A method of *in vitro* screening for one or more ligands from a group of test substances, said test substances being selected from the group consisting of estrogens and compounds having estrogen activity, said method comprising the steps of:

a) providing a cell-free or enzymatic assay system for each of said test substances, said cell-free or enzymatic assay system comprising an estrogen receptor for said test substances and a co-present steroid receptor coactivator-1, and fragments thereof;

b) experimentally determining half-maximally effective ligand concentrations ($EC_{50(ER+SRC)}$) for each of said test substances at which a physical-chemical interaction of said co-present steroid receptor coactivator-1, and said fragments thereof, and said estrogen receptor occurs in the cell-free or enzymatic system in the presence of each of said test substances; and

c) selecting said one or more ligands from said group of test substances if said half-maximally effective ligand concentration ($EC_{50(ER+SRC)}$) for said one or more ligands is greater than or equal to 100 nmol/l.

5. The method as defined in claim 4, wherein said physical-chemical interaction is detected by experimentally measuring fluorescence energy transfer between a

fluorescently-labeled steroid receptor coactivator-1 and a fluroescently-labeled nuclear receptor.

6. The method as defined in claim 4, wherein said one or more ligands are from said estrogens and transcriptionally activate a cellular assay system at half-maximally effective ligand concentrations less than or equal to 10 nmol/l, wherein said cellular assay system comprises said estrogen receptor and an estrogen-receptor-driven reporter gene.

7. A method of screening a group of test substances for one or more ligands to be administered as effective ingredients in a method of treating neuro-degeneration in cerebral cortex, said test substances being selected from the group consisting of estrogens and compounds having estrogen activity, said method of screening comprising the steps of:

a) providing a cell-free or enzymatic assay system for each of said test substances, said cell-free or enzymatic assay system comprising an estrogen receptor for said test substances and a co-present steroid receptor coactivator-1, and fragments thereof;

b) experimentally determining half-maximally effective ligand concentrations ($EC_{50(ER+SRC)}$) for each of said test substances at which a physical-chemical interaction of said co-present steroid receptor coactivator-1, and said fragments thereof, and said estrogen receptor occurs in the cell-free or enzymatic system in the presence of each of said test substances;

c) selecting said one or more of said test substances if said half-maximally effective ligand concentration ($EC_{50(ER+SRC)}$) for said one or more of said test substances is greater than or equal to 100 nmol/l;

d) providing a cellular or tissue assay system comprising an estrogen receptor and an estrogen receptor-driven reporter gene;

e) experimentally determining half-maximally effective ligand concentrations ($EC_{50(ER)}$) for said one or more test substances selected during the selecting of step c) at which said cellular or tissue assay system is transcriptionally activated in the presence of said one or more test substances; and

f) selecting those of said one or more test substances having said half-maximally-effective ligand concentrations that transcriptionally activate said cellular or tissue assay system and that are less than or equal to 10 nmol/l as said one or more ligands for said method of treating said neuro-degeneration in said cerebral cortex.

8. A method of treating neuro-degeneration in cerebral cortex of a human being, said method comprising the step of administering an effective amount of 3',15β-dihydrocycloprop[14,15]-estra-1,3,5(10),8-tetraene-3,17α-diol of said human being.

9. The method as defined in claim 7 or 8, wherein said neuro-degeneration is an age-related cognitive disorder, affective disorder, Alzheimer's disease or cerebral ischemia/stroke.

In the Abstract:

Please replace the original abstract with the following replacement abstract:

Abstract of the Disclosure

A method for *in vitro* screening a group of test substances for a ligand using two assay systems, i.e. a cellular or tissue assay system and an enzymatic assay system, is described. First, those test substances are selected which have transcriptional ER-mediated activity measured by an ER-driven reporter gene in the cellular or tissue assay system with an $EC_{50(ER)}$ (half-maximally effective ligand concentration) lower than or equal to 10 nmol/l. Then in an enzymatic assay system the selected test substances having the required transcriptional ER-mediated activity are tested by measuring a physical-chemical interaction (recruitment) of SRC-1 and the ER in the presence of the test substances. The selected ligand activates the ER and induces interaction with the co-present SRC-1 with an $EC_{50(ER+SRC)}$ higher than or equal to 100 nmol/l. The ligands found by the inventive screening method are useful for treatment and prevention of neuro-degeneration in the cerebral cortex, especially of age-related cognitive disorders, affective disorders, Alzheimer's diseases and cerebral ischemia/stroke.

REMARKS

This is a simultaneous amendment for the above-identified U.S. Patent Application.

First, claims 1 to 3 have been amended ***solely*** to eliminate wording that could be considered indefinite under 35 U.S.C. 112, second paragraph, and to put these claims in a form that complies somewhat better with U.S. Patent Office Rules for claim drafting.

More specifically, abbreviations, which obscure the claim meaning for some readers, have been eliminated and replaced by the appropriate terms from the specification. Changes have been made to provide antecedent basis for claim terms. Illogical wording has been eliminated. For example the terms "first" and "second" assay system are no longer used, because only one cell-free assay system and one cellular or tissue assay system are recited in the claims. However no changes, which might change the scope or substance of these claims, have been made.

The changes made in claims 1 to 3 are shown in the appendix herein-below. Also a somewhat simpler paragraph style has been used in the amended claims. The brackets showing deletions show deletion of some spaces as well as words so that the changes in the paragraph style are also shown in the appendix below to some extent.

In addition, new method claims 4 to 9 have been added. New independent method claim 4 is at least approximately of the same scope or breadth as amended claim 2. It is substantially reworded to describe the method in simpler terms in English. New claim 5 claims the embodiment for the method of detecting the physical-chemical interaction between SRC-1 and the ER that is disclosed in the specification, namely fluorescence-labeling. Claim 6 is similar to claim 3 and depends on claim 4. Claim 7 is an independent claim for a method of finding ligands useful for treatment of neuro-degeneration of the cerebral cortex. The specification and abstract provide basis for this claim. Independent claim 8 claims a preferred compound for treating neuro-degeneration of the cerebral cortex that is disclosed in the examples in the specification. Dependent claim 9 depends on claim 7 or 8 and states specific examples of neuro-degeneration that are treatable with the selected ligands.

The abstract has been amended so that it is in one paragraph and complies with that and other U.S. Patent Office Rules.

APPENDIX SHOWING THE CHANGES IN THE ORIGINAL CLAIMS REQUIRED TO OBTAIN THE ABOVE REPLACEMENT CLAIMS

Underlining shows additions, brackets show deletions

In the Claims:

1(amended). A method of [in vitro] *in vitro* screening for a [test substance (ligand)] ligand [involving] including selecting [and detecting] said ligand by means of at least two assay systems, said method comprising the [following] steps of:

[(i)] a) in a [first] cellular or tissue assay system comprising an estrogen receptor and an estrogen receptor-driven reporter gene, selecting the ligand [have] having a transcriptional activity [

which is] mediated by activation of [ER] the estrogen receptor and [

which is] measured by detecting a potency in the cellular or tissue assay system [comprising ER and an ER-driven reporter gene],

whereby [,]in the [first] cellular or tissue assay system the ligand activates the potency with [an $EC_{50(ER)}$ (half-maximally effective ligand concentration) lower] a half-maximally effective ligand concentration ($EC_{50(ER)}$) less than or equal to 10 nmol/l, and

detecting the activation of the transcription;

and

[(ii)] b) in a [second] cell-free or enzymatic assay system, selecting [the] a physical-chemical interaction [(recruitment) of SRC-1] of a co-present steroid receptor coactivator-1, and fragments thereof, [and the ER] and the estrogen receptor, [

] which is measured by detecting [the] a potency of [this] said interaction in the cell-free or enzymatic system,

wherein the ligand activates the [ER] estrogen receptor and induces said interaction with [the co - present SRC-1] said co-present steroid receptor coactivator-1, and fragments thereof, in the [second] cell-free or enzymatic assay system with [a $EC_{50(ER+SRC)}$ higher] a half-maximally effective ligand concentration ($EC_{50(ER+SRC)}$) greater than or equal to 100 nmol/l, and detecting [the] a potency of the physical-chemical interaction of [SRC-1] the co-present steroid receptor coactivator-1, and fragments thereof, [and of ER] and the estrogen receptor.

2(amended). A method of [in vitro] *in vitro* screening for a [test substance (ligand)] ligand, [

] which is a known estrogen or a] said ligand being an estrogen or having [with] estrogenic activity, in a cell-free or enzymatic assay system by selecting [the] a physical-chemical interaction of a co-present steroid receptor coactivator-1 [(recruitment) of SRC-1], and fragments thereof, and [of the ER] an estrogen receptor, [

which is] said physical-chemical interactoin being measured by detecting [the] a potency of [this] said interaction in the cell-free or enzymatic assay system,

wherein the ligand activates the [ER] estrogen receptor and induces said interaction with the co-present [SRC-1] steroid receptor coactivator-1, and fragments thereof, in the [second] cell-free or enzymatic assay system with a [$EC_{50(ER+SRC)}$ higher] half-maximally effective ligand concentration ($EC_{50(ER+SRC)}$) greater than or equal to 100 nmol/l, and detecting the potency of the physical-chemical interaction of [SRC- 1] said co-present steroid receptor coactivator-1, and fragments thereof, and of [ER] said estrogen receptor.

3. A method of [an] *in vitro* [in vitro] screening [for a test substance (ligand)] according to claim 2, wherein said [

which] ligand is [an] said estrogen and transcriptionally activates a cellular assay system comprising [ER and an ER-driven] said estrogen receptor and an estrogen-receptor-driven reporter gene, [

] wherein the ligand activates [the] a potency with a half-maximally effective ligand concentration ($EC_{50(ER)}$) [an $EC_{50(ER)}$ (half-maximally effective ligand concentration) lower] less than or equal to 10 nmol/l.

In the Abstract:

Abstract of the Disclosure

A method [of an in vitro] for *in vitro* screening of a group of test substances for a ligand using two assay systems, i.e. [in] a [first] cellular or tissue assay system and an enzymatic assay system, is described. [, selecting the ligand with] First, those test substances are selected which have transcriptional ER-mediated activity measured by an ER-driven reporter gene [, whereby] in the [first] cellular or tissue assay system [the ligand activates the potency] with an $EC_{50(ER)}$ (half-maximally effective ligand concentration) lower than or equal to 10 nmol/l. [, and,] Then in [a second] an enzymatic assay system the selected test substances having the required transcriptional ER-mediated activity are tested by measuring a [, selecting the] physical-chemical interaction (recruitment) of SRC-1 and the ER in the presence of the test substances. [, wherein the] The selected ligand activates the ER and induces interaction with the co-present SRC-1 with an $EC_{50(ER+SRC)}$ higher than or equal to 100 nmol/l. [

] The ligands found by the inventive screening [can be used] method are useful for [the] treatment and prevention of neuro-degeneration in the cerebral cortex, especially [and are thus useful for treatment and prevention] of age-related cognitive disorders, affective disorders, Alzheimer's diseases and cerebral ischemia/stroke.

Should the Examiner require or consider it advisable that the specification, claims and/or drawing be further amended or corrected in formal respects to put this case in condition for final allowance, then it is requested that such amendments or corrections be carried out by Examiner's Amendment and the case passed to issue. Any costs involved should be charged to the deposit account of the undersigned (No. 19-4675). Alternatively, should the Examiner feel that a personal discussion might be helpful in advancing the case to allowance, he or she is invited to telephone the undersigned at 1-631-549 4700.

In view of the foregoing, favorable allowance is respectfully solicited.

Respectfully submitted,

Michael J. Striker,

Attorney for the Applicants

Reg. No. 27,233